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# Appearance matters: sedimentation effects on different sponge morphologies

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*Dredging activity poses an environmental risk to sponges as sediments from the dredge or disposal site may smother the sponge surface, potentially affecting water filtration and light penetration. Dredge-related sedimentation effects may also vary between sponge morphologies, potentially impacting community structure and functioning. To test this, 10 sponge species encompassing four different morphologies (massive, erect, cup and encrusting), were exposed to a single pulse treatment of three different sediment concentrations (0, 250 and 500 mg $l^{-1}$) and followed over 2 weeks, in 1000 l tanks. Total suspended solids (TSS) and sedimentation rates (SR) were recorded throughout the study. A sharp decrease in TSS was recorded within the first 2–3 h and a total settlement of sediments occurred within the first 48 h of the pulse exposure (0, 8 and 16 mg $cm^{-2}$ in the control, medium and high sediment treatments, respectively). The effects of high sedimentation included mortality of cup-shaped* Callyspongia confoederata *and small areas of tissue necrosis in other species, with massive, encrusting and wide cup morphologies particularly affected. However, the sediment concentrations tested in this experiment did not cause changes in the concentration of sponge pigments or the structure of the symbiotic microbial community in any species. These results indicate that a single pulse of sediments less than 16 mg $cm^{-2}$ is not detrimental to most of the sponge species studied.*

**Keywords:** Australia, chlorophyll, dredging, sedimentation, sponge, symbiont

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## INTRODUCTION

Dredging of the sea bed is required for the development and maintenance of harbours and offshore petrochemical facilities around the world (Morton, 1977; DEWHA, 2009). However, this activity requires the removal and subsequent dumping of millions of cubic metres of spoil into proximate areas, posing an environmental risk for marine communities (Morton, 1977; Desprez, 2000). One of the main physical effects of dredging is the temporary increase in suspended sediment concentrations at both dredge and disposal sites (Morton, 1977). Dredged sediments can remain in suspension for minutes to days depending on their particle size and composition, and local environmental conditions, before settling on the seafloor and benthic communities (Newell *et al.*, 1998). Increased sedimentation and levels of total suspended solids (TSS) are considered major causes of worldwide degradation of important marine ecosystems such as coral reefs (Rogers, 1990; McClanahan & Obura, 1997; McCulloch *et al.*, 2003), rocky assemblages (Airoldi, 2003; Balata *et al.*, 2005) and estuaries (Wilber & Clarke, 2001). The effects of high sedimentation and TSS range from the immediate burial and smothering of benthic organisms to negative effects on life history processes such as settlement, recruitment, feeding and growth (Airoldi, 2003; Fabricius, 2005; Lohrer *et al.*, 2006). Competitive and predator-prey interactions may also be affected by high sedimentation (Airoldi, 2003).

Sponges are sessile filter-feeding organisms that play important roles in marine ecosystems including occupying and eroding substrate, bentho-pelagic energy transfer, and positive and negative associations with other organisms (Bell, 2008; de Goeij *et al.*, 2013). Sponges are highly diverse and can even be the dominant fauna in many regions including some coral reefs, inter-reef habitats and deep water environments (e.g. Wilkinson & Evans, 1989; Bell & Barnes, 2000a; Diaz & Rützler, 2001; Pawlik, 2011; Murillo *et al.*, 2012). In areas of particularly high sponge abundance (sponge gardens), these organisms can fulfil ecological roles comparable to coral reefs (Schönberg & Fromont, 2011). However, sponge assemblages can also be sensitive to global and local pressures including climate change (Przeslawski *et al.*, 2008; Bell *et al.*, 2013; Webster *et al.*, 2013) and dredging-associated increases in sediment suspension and deposition (Gerrodette & Flechsig, 1979; Wilkinson & Cheshire, 1989; Roberts *et al.*, 2006; Bannister *et al.*, 2012).

The effects of sediments on sponges include clogging of the aquiferous canals and chambers (Bakus, 1968), reduced pumping activity (Gerrodette & Flechsig, 1979; Tompkins-MacDonald & Leys, 2008), reduced growth rates (Roberts *et al.*, 2006; Whalan *et al.*, 2007) and increased respiration rates (Bannister *et al.*, 2012). High sediment deposition can also smother sponge recruits (Maldonado *et al.*, 2008) and even bury adults (Wulff, 1997). In addition, sponges host dense and diverse microbial symbionts that contribute to the health, fitness and nutrition of the host (Webster & Taylor, 2012). Many of these symbionts are photosynthetic which may make sponges particularly sensitive to

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dredge-related light reduction due to increased turbidity (Thacker, 2005; Roberts *et al.*, 2006; Bell, 2008). High sedimentation can therefore greatly influence the structure, abundance and diversity of sponge assemblages (Bell & Barnes, 2000a, b; Carballo, 2006).

Effects of sedimentation will vary between sponge species with morphology or shape likely to be a major contributor to this inter-species variation. For example, high sediment deposition may smother thin, encrusting sponges while having little impact on upright or erect sponges. The overall aim of this study was to investigate responses of different sponge morphologies to a range of controlled sedimentation treatments simulating conditions associated with dredging activity.

## MATERIALS AND METHODS

### Sponge species and morphology

Ten sponge species (Table 1), representing four general morphologies (massive, erect, cup and encrusting), were collected from Broadhurst Reef, Great Barrier Reef (18°51.891′S 147°41.660 E), in September 2013. Of these, *Stylissa flabelliformis*, *Cliona orientalis*, *Ianthella basta* and *Carteriospongia foliascens* are also widely distributed throughout the Indo-Pacific (Fromont, 2004). Sponges were transported to the Australian Institute of Marine Science and acclimated in 1000 l tanks for >2 weeks with 5 μm filtered flow-through seawater at 25°C and 36‰ salinity, environmental conditions comparable to the collection site.

### Experimental set up

The experiment was conducted in three 1000 l tanks, each dosed at one of three sediment levels: 0 mg $l^{-1}$ (control), 250 mg $l^{-1}$ (medium) and 500 mg $l^{-1}$ (high). The siliciclastic sediment used in this experiment was collected sub-tidally from Onslow, Western Australia (21°38′S 114°56′E) and ground to 63 μm. Particle size distribution analysis determined that the ground sediment ranged from 1–130 μm in size, with the majority between 30 and 80 μm. Heavy metal analysis determined that the grinding process did not contaminate sediments but that they were naturally rich in Fe and Al.

For the medium and high treatments, ground sediment was blended with seawater, forming a sediment slurry that was poured slowly into each tank in a single pulse at Day 0 to reach desired treatment concentrations. Water flow in the tanks was standardized to ~300 ml $min^{-1}$, resulting in a complete renewal of seawater in 48 h.

One to four replicates of each sponge species were randomly placed in each tank, with all sponges separated by ≥10 cm to prevent any antagonistic interactions. All species were placed in their natural orientation. For example, *I. basta*, *Haliclona* sp., *S. flabelliformis* and *Callyspongia confoederata* were fixed to coral plugs using non-toxic underwater putty (Knead IT® Aqua Selleys, NSW Australia) and placed in plastic racks, so that they were exposed to sediments in their natural upright position. Sponges were exposed to sediments for 15 days.

No significant differences in initial size of replicates among treatments were observed for any of the species (ANOVA: $P > 0.05$ for all species), thus excluding initial sponge size influencing the results.

### Studied parameters

#### PHYSICAL PARAMETERS

Total Suspended Solids (TSS) were recorded immediately following sediment addition ($T = 0$ h), $T = 8$, 24 and 32 h, and then after 7 and 14 days. For TSS analysis, triplicate water samples (200 ml) were collected from each tank and filtered through previously weighed 0.4 μm polycarbonate filters (Advantec MFS, Inc.). Filters were dried overnight at 60°C and dry weights were recorded the following morning.

Sedimentation Rates (SR) were measured by collecting and filtering sediments that accumulated on five SedPods (Surface Area = 25.16 $cm^2$) (Field *et al.*, 2013) randomly placed in each tank. To examine sedimentation rates throughout the experiment, SedPods were removed after 1, 2, 7 and 15 days.

#### DETERMINATION OF SMOTHERING AND ASSESSMENT OF SPONGE HEALTH

Underwater pictures of each sponge were taken with an Olympus C-5050 digital camera prior to sediment addition and after 2, 7 and 15 days. Image analysis software (Image J) was then used to measure the percentage of surface area covered by sediments for each sponge during the experiment (Figure 1).

To determine the weight of sediment remaining on each sponge by the end of the experiment, each sponge was carefully placed into a plastic zip lock bag underwater, and then inverted and shaken so that all sediment fell off the sponge

**Table 1.** List of species, morphologies and number of replicates per treatment.

| Species name | Functional morphology | Replicates |
|---|---|---|
| *Callyspongia confoederata* (*sensu* Ridley, 1884) | Cup (narrow cup or tube) | 2 |
| *Carteriospongia foliascens* (Pallas, 1766) | Cup (wide cup) | 2 |
| *Cliona orientalis* Thiele, 1900 | Encrusting (bioeroding) | 3 |
| *Cymbastela coralliophila* Hooper & Bergquist, 1992 | Encrusting (thick)/Cup (table) | 2 |
| *Haliclona* sp. Grant, 1836 | Cup (narrow cup or tube) | 2 |
| *Ianthella basta* (Pallas, 1976) | Erect (laminar) | 2 |
| *Ircinia irregularis* (Polejaeff, 1884) | Massive (simple) | 1 |
| *Neopetrosia exigua* (Kirkpatrick, 1900) | Massive/Encrusting | 2 |
| *Rhopaloeides odorabile* Thompson, Murphy, Bergquist & Evans, 1987 | Massive (simple) | 4 |
| *Stylissa flabelliformis* (Hentschel, 1912) | Erect (laminar) | 2 |

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**Fig. 1.** Tested sponge species prior to the sediment addition (before), during the experiment (during) and after removal of sediments (after) in the high sediment treatment: (A) *R. odorabile*; (B) *I. irregularis*; (C) *N. exigua*; (D) *I. basta*; (E) *S. flabelliformis*; (F) *Haliclona* sp.; (G) *C. confoederata*; (H) *C. foliascens*; (I) *C. orientalis*; (J) *C. coralliophila*. Scale bars: 1 cm.

surface into the bag. The water and sediment within each plastic bag was filtered and weighed as described above.

Following the removal of sediments and any attached algae, additional pictures were taken of each sponge to confirm sponge mortality, determine colour changes and calculate the percentage of necrosed tissue. The change in sponge surface area from day 0 to day 15 was measured for each sample to determine approximate sponge growth (Figure 1). Importantly however, inferred growth based on surface area could be underestimated, especially in sponges with a three-dimensional structure such as cups and erect morphologies.

Changes in surface area (i.e. growth), percentage of necrosed tissue, percentage of area covered by sediments on day 2 and total sedimentation on sponges were studied separately for those species with sufficient levels of replication to enable statistical testing (i.e. ≥3 replicates: *Rhopaloeides odorabile* and *C. orientalis*) with a one-way analysis of variance (ANOVA) using treatment as the fixed factor. For all four variables, we performed a two-way general linear model (GLM) ANOVA with sponge morphology (as per Table 1) and treatment as fixed factors. Logit and arcsine transformations were performed to meet the assumptions for ANOVA. Transformed data had homogeneity of variances in all datasets, although in some instances normality was not accomplished. GLM ANOVA tests were still conducted as they are robust to departures from normality when variances are homogeneous (Underwood, 1997). Statistical analysis and graphs were performed using the software SigmaPlot v.11.0 (Systat Software Inc.) and NCSS v 9 (NCSS, USA).

### PIGMENT ANALYSIS

At the completion of the experiment, two 1 × 0.5 × 0.5 cm pieces of healthy tissue per sponge individual were excised using sterile scalpels, cutting from the pinacoderm through to the mesohyl. Excised pieces were briefly rinsed in clean seawater to remove surface sediments, placed into two 2 ml cryo-vials and snap frozen in liquid nitrogen for subsequent analysis of pigments and microbial symbionts.

Chlorophyll and carotenoid concentrations were used as a proxy for host stress and photosynthetic potential after exposure to sediments. Samples were allowed to thaw slightly and approximately 0.25 g wet weight of each sample was finely cut and extracted in 2 ml of 95% ethanol. Three stainless steel beads were added to each vial, and samples were shaken in a Bead Beater (Bio Spec Products Inc., Bartlesville, USA) for 3 min. Triplicate 300 µl extracts, and the 95% ethanol blank, were then pipetted into a microplate.

The extraction method using 95% ethanol was less toxic and more time efficient than extractions with acetone or methanol and also yielded greater extraction concentrations (data not shown). As ethanol is less volatile than methanol or acetone, it can be used in 96-well microplates for assessment using the spectrophotometer.

Absorbance at 470, 632, 649, 665, 696 and 750 nm (i.e. turbidity) was read on a Power Wave Microplate Scanning Spectrophotometer (BIO-TEK® Instruments Inc., Vermont, USA). Using the blank corrected absorbance readings minus the absorbance at wavelength 750 nm ($E_x$), Chl *a*, Chl *b*, Chl *c*, Chl *d*, Total Chl and Total Carotenoids were calculated using the following equations (Lichtenthaler, 1987; Ritchie, 2008):

$$\text{Chl } a\,(\mu\text{g ml}^{-1}) = [(-0.9394 \times E_{632}) + (-4.2774 \times E_{649}) + (13.3914 \times E_{665})]/0.794$$

$$\text{Chl } b\,(\mu\text{g ml}^{-1}) = [(-4.0937 \times E_{632}) + (25.6865 \times E_{649}) + (-7.3430 \times E_{665})]/0.794$$

$$\text{Chl } c\,(\mu\text{g ml}^{-1}) = [(28.5073 \times E_{632}) + (-9.9940 \times E_{649}) + (-1.9749 \times E_{665})]/0.794$$

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$$\text{Chl } d\ (\mu\text{g ml}^{-1}) = [(-0.2007 \times E_{632}) + (0.0848 \times E_{649}) + (-0.1909 \times E_{665}) + (12.1302 \times E_{696})]/0.794$$

$$\text{Total Chl } (\mu\text{g ml}^{-1}) = [(24.1209 \times E_{632}) + (11.2884 \times E_{649}) + (3.7620 \times E_{665}) + (5.8338 \times E_{696})]/0.794$$

$$\text{Total carotenoids } (\mu\text{g ml}^{-1}) = [((1000 \times E_{470})/0.794) - (2.13 \times \text{Chl } a) - (97.64 \times \text{Chl } b)]/209$$

The factor 0.794 is a path length correction, determined by the ratio of the absorbance of the microplate measurement divided by the absorbance of the 1 cm cuvette at a given wave length, using ethanol as solvent and for a volume of 300 µl of sample extract.

Pigment concentrations were normalized to wet weight using the calculation:

$$[\text{Chl } a\ (\mu\text{g ml}^{-1}) \times \text{extraction volume (ml)}]/\text{wet weight (g)}$$

### MICROBIAL SYMBIONTS ANALYSIS

Assessment of host-associated microbial communities was performed for all sponge species that had ≥2 replicates alive in all treatments at the completion of the experiment (i.e. *C. foliascens*, *C. orientalis*, *Cymbastela coralliophila*, *I. basta*, *Neopetrosia exigua*, *R. odorabile* and *S. flabelliformis*). DNA extractions were performed using the Power Plant® Pro DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA) and a fragment of the 16S rRNA gene was amplified with the primer set 1055F and 1392r containing a GC clamp (Muyzer *et al.*, 1993; Ferris *et al.*, 1996). Total reaction volume was 50 µl, including 10 µl of 5× Buffer (containing 5 mM dNTPs and 15 mM $MgCl_2$), 0.4 µl of BSA (10 mg ml$^{-1}$), 0.25 µl (1.25 units) of My *Taq* DNA Polymerase (Bioline®, London, UK), 1 µl of each primer (10 µM), ~10 ng of template DNA and sterile Milli-Q water. PCR conditions were as follows: 1 cycle at 95°C for 1 min; 32 cycles at 94°C for 30 s, 54°C for 30 s and 72°C for 1 min, and a final elongation at 72°C for 7 min. PCR products were visualized on 1% agarose gels to assess amplification specificity and initial product quantity. 15 µl of each PCR product were applied to 8% w/v polyacrylamide (37.5:1) gels containing a 50–70% denaturing gradient of formamide and urea. Gels were electrophoresed at 65°C for 16 h in 1 × TAE (Tris-acetic acid EDTA) buffer at 75 V using the Ingeny D-Code system (Goes, the Netherlands). Gels were stained with 1× Sybr Gold for 10 min, visualized under UV illumination and photographed. Individual band numbers were assigned based on their migration. Bands assigned the same number had identical migration end points, and were used to build a presence/absence matrix. Three factors were determined (i.e. species, morphology, sediment treatment). principal component analysis (PCA) of microbial community profiles was performed on square root transformed data. The same matrix was used for SIMPER analysis (similarity/distance percentages), which examined the contribution of each variable to average resemblances between sample groups. A distance matrix was obtained using Bray–Curtis similarity and used for PERMANOVA (Permutational multivariate ANOVA based on distances). All analyses were performed on Primer 6 (PRIMER-E Ltd, Plymouth, UK).

## RESULTS

### Physical parameters

Levels of TSS measured at Time 0 (directly after addition of sediments to the tanks) were 1.8 ± 0.3, 217 ± 7 and 542 ± 87 mg l$^{-1}$ (mean ± SE) in the control, medium and high treatment tanks, respectively. Thus, measured TSS values were close to expected values of 0, 250 and 500 mg l$^{-1}$. TSS dropped ~80% in 8 h and 99% in 48 h in both treatment tanks.

Total sedimentation levels at the end of the experiment were 0.27 ± 0.01, 8.7 ± 0.7 and 16.3 ± 0.3 mg cm$^{-2}$ (mean ± SE) in the control, medium and high treatment tanks, respectively. Similar to the TSS levels, a ~98% decline in SR was observed within 24 h in both sediment treatments with all sediments settling within 48 h. The primary main physical effect on sponges in this study was therefore sediment deposition.

### Determination of smothering and assessment of sponge health

With the exception of individuals of *Haliclona* sp. and *Callyspongia confoederata*, all sponges in all treatments survived until the end of the experiment. *Haliclona* sp. died after 7 days in the medium and high sedimentation treatments and after 15 days in the control, indicating that this species is unsuitable for further aquarium-based experimentation. In contrast, *C. confoederata* survived in the control treatment, but died after 7 days in the high sediment treatment and after 15 days in the medium sediment treatment, indicating sensitivity to elevated sediment levels (Figure 1).

With the exception of *Ianthella basta*, *Haliclona* sp. and *C. confoederata*, all species showed positive growth (i.e. surface area) in the control treatment (Figure 2). In contrast, null or negative growth was observed in the medium and high sediment treatment for all species except *Cliona orientalis* which exhibited positive growth in the medium treatment but negative growth in the high treatment (Figure 2). Although differences in growth between treatments were not statistically significant for *Rhopaloeides odorabile* and *C. orientalis* (ANOVA: $P > 0.05$), both morphology and treatment had a significant effect on growth when comparing all individuals grouped by morphology (Table 2). Massive and encrusting species showed a significantly higher growth than cups and erect sponges. Sponges exposed to medium and high sediment treatments grew significantly less than sponges in the control treatment (Table 2, Figure 2).

Individuals of some species in the medium and high sediment treatments developed areas of necrosed tissue (e.g. 2–15% of *R. odorabile* tissue and 2.6–5% of *Cymbastela coralliophila* tissue) although there was no significant difference between treatments (ANOVA: $P > 0.05$). Tissue necrosis

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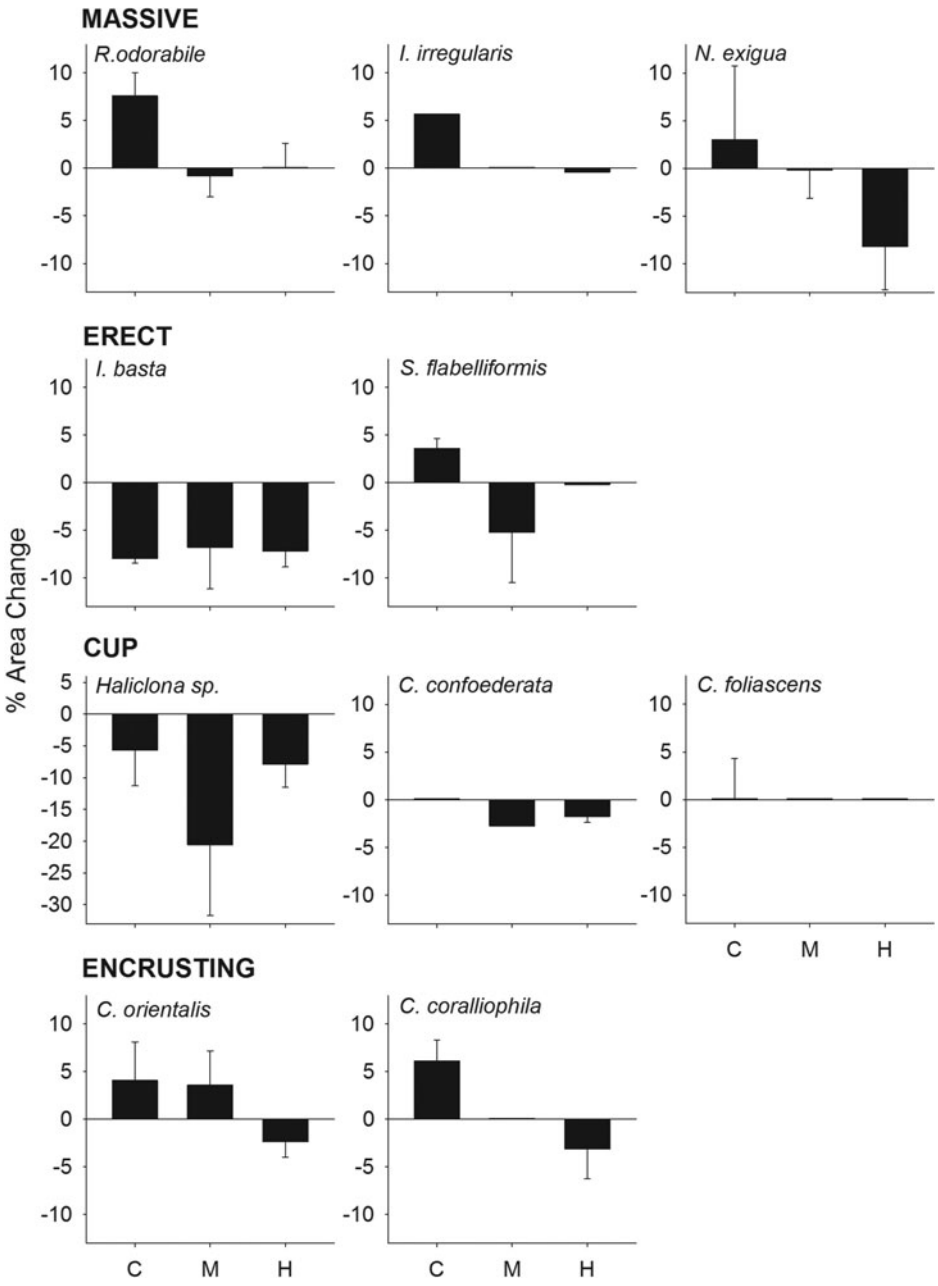


**Fig. 2.** Percentage of area change (growth) at the end of the experiment for all species grouped by morphologies, at the three sediment treatments (control, medium and high).

was not observed in any individual of the erect species *Stylissa flabelliformis* and *I. basta* and the encrusting species *C. orientalis*. Significant differences in percentage of necrosis were only observed when comparing among morphologies, with cups experiencing the highest percentage of necrosis (Table 3, Figure 1).

**Table 2.** ANOVA examining the effects of treatment on size (growth) among the sponge morphologies after 15 days.

| Source | *df* | MS | *F* | *P* |
|---|---|---|---|---|
| Morphology | 3 | 0.0198 | 4.034 | 0.012 |
| Treatment | 2 | 0.0172 | 3.511 | 0.037 |
| Morphology × Treatment | 6 | 0.00478 | 0.974 | 0.452 |
| Error | 53 | 0.00491 | | |

Significant Pairwise Multiple Comparisons (Holm–Sidak method).
MAS, ENC > CUP ($P = 0.009, 0.010$).
C > M ($P = 0.017$), H ($P = 0.025$).

**Table 3.** ANOVA examining the effects of treatment on the percentage of tissue affected by necrosis among the sponge morphologies after 15 days.

| Source | *df* | MS | *F* | *P* |
|---|---|---|---|---|
| Morphology | 3 | 2.964 | 18.027 | <0.001 |
| Treatment | 2 | 0.301 | 0.301 | 0.301 |
| Morphology × Treatment | 6 | 0.126 | 0.769 | 0.598 |
| Error | 53 | 0.164 | | |

Significant Pairwise Multiple Comparisons (Holm–Sidak method).
CUP > ENC ($P = 0.009$), MAS ($P = 0.010$), ERE ($P = 0.013$).

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**Table 4.** ANOVA examining the differences in percentage of sponge surface covered by sediments among the sponge morphologies after 48 h.

| Source | *df* | MS | *F* | *P* |
|---|---|---|---|---|
| Morphology | 3 | 0.255 | 5.346 | 0.003 |
| Treatment | 2 | 2.149 | 45.016 | <0.001 |
| Morphology × Treatment | 6 | 0.103 | 2.163 | 0.061 |
| Error | 53 | 0.0477 | | |

Significant Pairwise Multiple Comparisons (Holm–Sidak method).
MAS > CUP ($P = 0.009$), ERE ($P = 0.010$).
C < M ($P = 0.025$), H ($P = 0.017$).

The percentage of sponge surface covered by sediments after 48 h differed significantly between treatments (ANOVA: $P < 0.001$, 0.03 for *R. odorabile* and *C. orientalis*, respectively) and morphologies (Table 4, Figure 3), with massive species having a greater coverage of sediments than cup and erect species. Nevertheless, the percentage of sponge surface covered by sediments decreased over time for most species, indicating an ability to remove some sediment from their surface tissue (Figure 4). This ability differed between species with *R. odorabile* removing less than 20% of the surface sediment and *Ircinia irregularis* removing 85% of the surface sediment after 15 days (Figure 4).

Total sedimentation onto sponges was significantly higher in the sediment treatments than the control (ANOVA: $P < 0.001$, 0.04 for *R. odorabile* and *C. orientalis*, respectively) and also differed significantly between the four main sponge morphologies (Figure 5, Table 5). Erect species received the

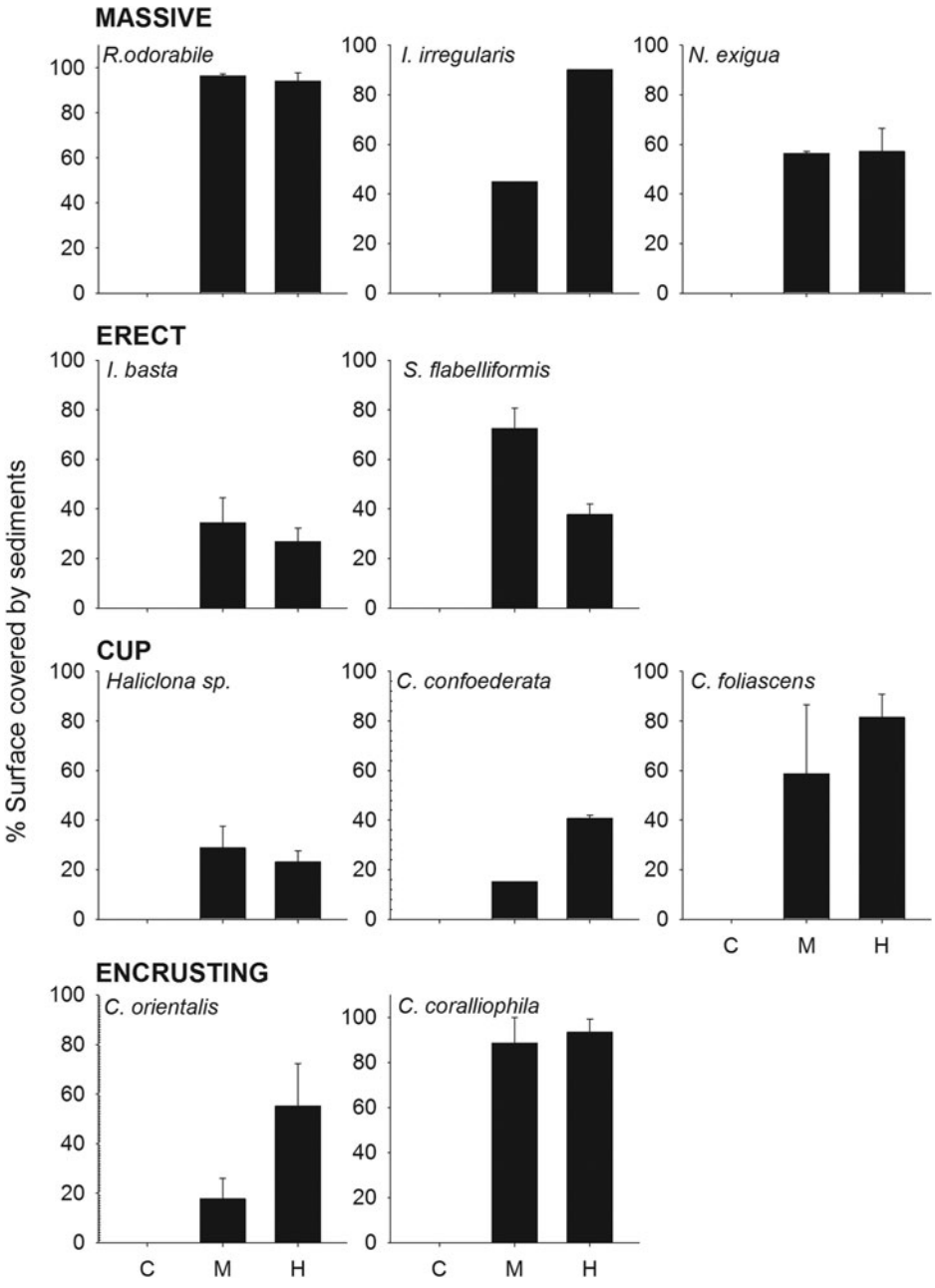


**Fig. 3.** Percentage of sponge surface covered by sediments 2 days after the sediment pulse for all species, grouped by morphologies, at the three sediment treatments (control, medium and high).

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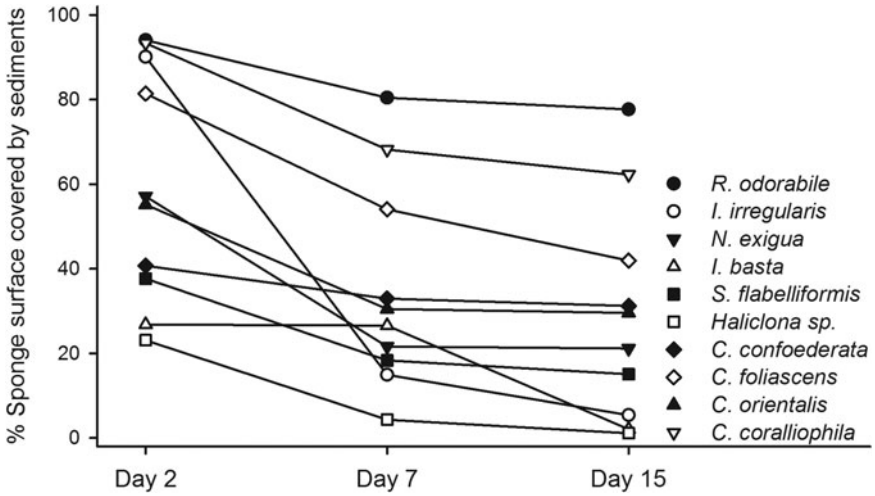


**Fig. 4.** Mean percentage of surface covered by sediments at day 2, 7 and 15 after sediment addition, for each sponge species at the high sediment treatment.

minimum amount of deposited sediment while cup-shaped sponges, particularly those with a wide cup morphology, collected the highest amount of sediment (Figure 5, Table 5). No correlation existed between total sedimentation and sponge tissue surface area covered by sediments ($R^2 = 0.218$, $P = 0.022$).

## Pigment analysis

Due to high rates of mortality, pigment concentrations were not analysed for *Haliclona* sp. and *C. confoederata*. For the remaining species, the concentration of photosynthetic pigments did not differ significantly between treatments (ANOVA: $P > 0.05$ for all species). However, pigment concentrations and types varied between species and treatments. For example, *R. odorabile* had an extremely low concentration of chlorophylls and carotenoids, consistent with the absence of photosymbionts previously described for this species (Bannister *et al.*, 2011) whereas *I. basta* and *S. flabelliformis* had low concentrations of chlorophylls, but high concentrations of carotenoids that decreased slightly with increasing sedimentation (Figure 6). The relatively high concentrations of Chl *a* in *C. orientalis* did not vary among treatments, while Chl *b* in this species was reduced slightly in the high sediment treatment (Figure 6). A slight increase in chlorophyll concentration in the sediment treatment compared with the control was observed in *I. irregularis* and *Neopetrosia exigua* (Figure 6). In general, concentrations of photosynthetic pigments were not reduced by the increasing sediment concentrations, although every species and every pigment responded differently.

## Microbial symbiont analysis

Microbial community profiling of replicate samples from *Carteriospongia foliascens*, *C. orientalis*, *C. coralliophila*, *I. basta*, *N. exigua*, *R. odorabile* and *S. flabelliformis* revealed 44 unique bands (corresponding to different microbial symbionts). The microbial profiles grouped according to species although some minor clustering according to sediment treatment was observable for *C. foliascens*, *R. odorabile* and *C. coralliophila* (Figure 7). The first two factors in the principal component analysis (PCA) explained 39.3% of the total variation (Figure 7). SIMPER analysis indicated high levels of similarity within species and moderate to high levels of similarity within morphologies: ≥50% for erect and encrusting, and ≥80% for massive and cup morphologies. PERMANOVA analysis of the microbial profiles revealed that microbial communities were significantly affected by host species and host morphology, but not by sedimentation treatment (Table 6).

# DISCUSSION

Rapid deposition of sediments after an initial sediment pulse combined with a rapid drop in TSS within 48 h indicates that the primary pressures on sponges in this study were sediment covering the surface tissue or clogging of their aquiferous system. Although the tested sedimentation treatments are consistent with values observed near dredging operations (e.g. 300 mg $l^{-1}$; Simpson, 1988), these sedimentation levels did not cause mortality during the 2-week experiment, except for *Callyspongia confoederata*. However, low levels of replication for this species precluded chlorophyll and symbiont analyses, making it difficult to reach definitive conclusions regarding the nature of the sediment sensitivity. Colour changes, generally indicating loss of photosynthetic symbionts or bleaching (Thacker, 2005; Roberts *et al.*, 2006), were not detected in any of the sponges that survived the experiment. Bleaching due to light attenuation may have occurred if there had not been such a rapid decrease in TSS or if the sediments had completely smothered the sponges.

All sponge morphologies shrunk when exposed to the high sediment treatment. Decreased or negative growth is likely linked to reduced feeding efficiency due to sediments clogging the sponge aquiferous systems (Gerrodette & Flechsig, 1979; Tompkins-MacDonald & Leys, 2008). For example, the glass sponge *Rhabdocalyptus dawsoni* arrests pumping entirely in response to sediments (Tompkins-MacDonald & Leys, 2008), the pumping rate of *Veronia lacunosa* has been shown to reduce with a sediment load as low as 11 mg $l^{-1}$ (Gerrodette & Flechsig, 1979) and similar responses have previously been observed in other species of demosponges

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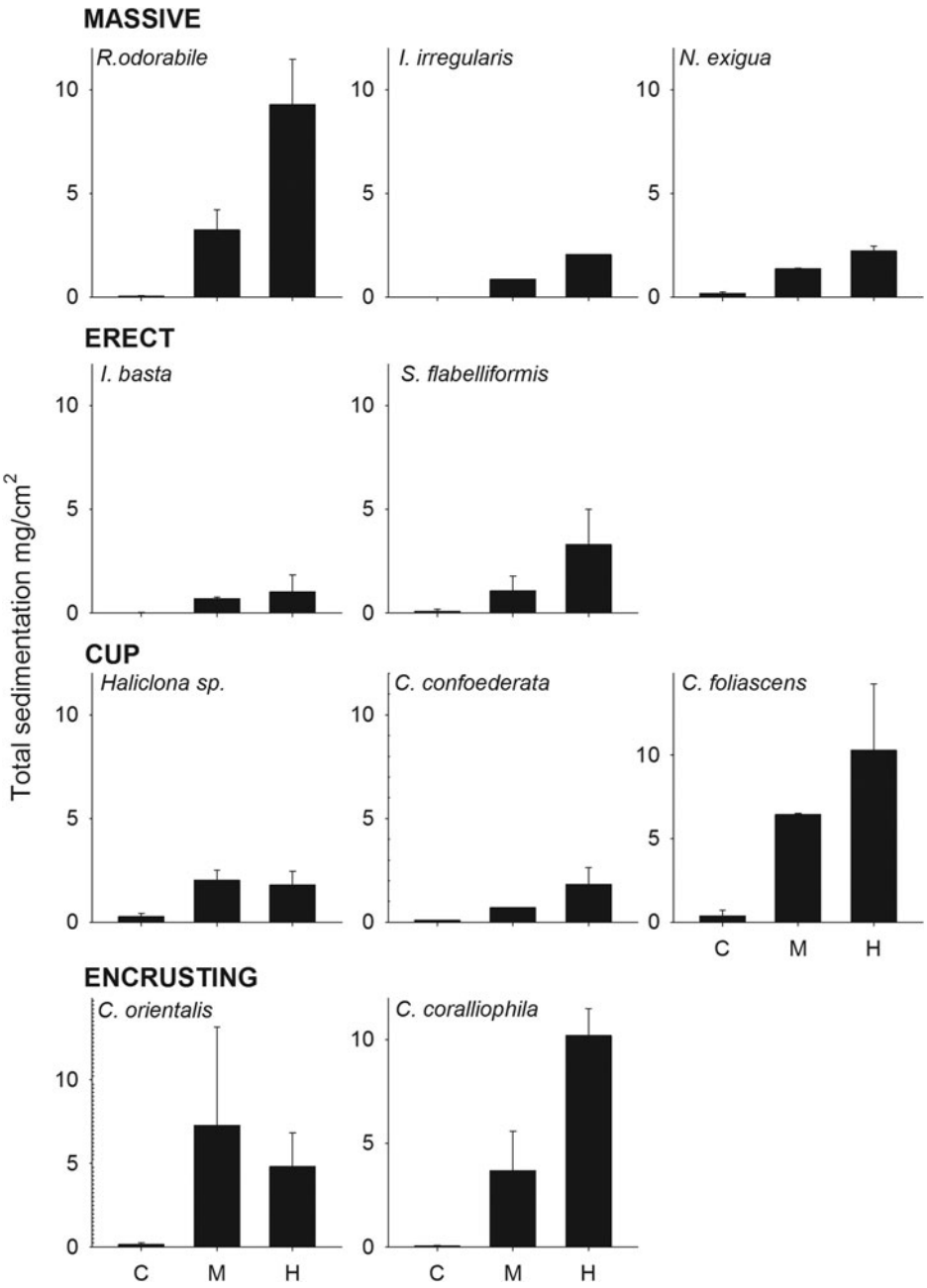


**Fig. 5.** Total sedimentation (mg $cm^{-2}$) at the end of the experiment for all species grouped by sponge morphology, at the three sediment treatments (control, medium and high).

(Reiswig, 1971; Lohrer *et al.*, 2006). Additionally, sponges living under high sediment conditions can become energetically stressed with efforts to expel unwanted material, contributing to a depletion of their reserves in comparison to individuals inhabiting areas less affected by sediment (Roberts *et al.*, 2006; Tompkins-MacDonald & Leys, 2008; Bannister *et al.*, 2012).

Notable differences in the total amount of entrapped sediments and the percentage of sponge surface covered by sediments were observed between the different sponge species and sponge morphologies. In general, massive, encrusting and wide cup morphologies accumulated more sediment than erect species and the narrow cup morphology. This is consistent with what has previously been reported for other sponge species (Gerrodette & Flechsig, 1979; Bell & Barnes, 2000b; Carballo, 2006). Although specific mechanisms were not elucidated within the context of this study, the reduction in sediment covering the sponges over time suggests that either the sponges are actively removing the sediment from their surface (i.e. through their pumping activity, production of mucus, protrusion of spicules) or other in-fauna organisms are doing it for them. For instance, *Iricinia irregularis* in this study had many brittle stars living inside their oscula, which may have indirectly cleaned their surfaces as found for other species (e.g. Hendler, 1984; Turon *et al.*, 2000).

The weak correlation between total sedimentation and sponge area covered by sediments could be explained by the presence of algae overgrowing some of the sponge individuals

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**Table 5.** ANOVA examining the differences in total sedimentation among the sponge morphologies after 15 days.

| Source | *df* | MS | *F* | *P* |
|---|---|---|---|---|
| Morphology | 3 | 5.747 | 4.109 | 0.011 |
| Treatment | 2 | 101.757 | 72.757 | <0.001 |
| Morphology × Treatment | 6 | 1.282 | 0.917 | 0.490 |
| Error | 53 | 1.399 | | |

Significant Pairwise Multiple Comparisons (Holm–Sidak method).
CUP > ENC ($P = 0.049$) > MAS ($P = 0.025$) > ERE ($P = 0.013$).
C < M ($P = 0.025$), H ($P = 0.017$).

by the end of the experiment. Algae attached to the surface of some sponges may have immobilized the sediments, making it difficult to remove and quantify the sediments. This would explain the observed underestimation in total sedimentation and highlights the critical importance of controlling algal growth in future experiments.

Chlorophyll and carotenoid analysis provided valuable data on pigment concentrations in each of the target sponge species. A reduction in Chl *a* has previously been reported in 90-days sediment-exposed (Roberts *et al.*, 2006) and 2 weeks-shaded sponges (Thacker, 2005), although these results were not statistically significant. However, elevated sediment concentrations did not appear to affect the production of any photosynthetic pigments in sponges over the 15-day exposure period. Moreover, the results were extremely variable among species and between pigments and no discernible trends in pigment behaviour could be determined according to sediment treatment. Therefore, the analyses of chlorophylls and carotenoids did not appear to be a valid

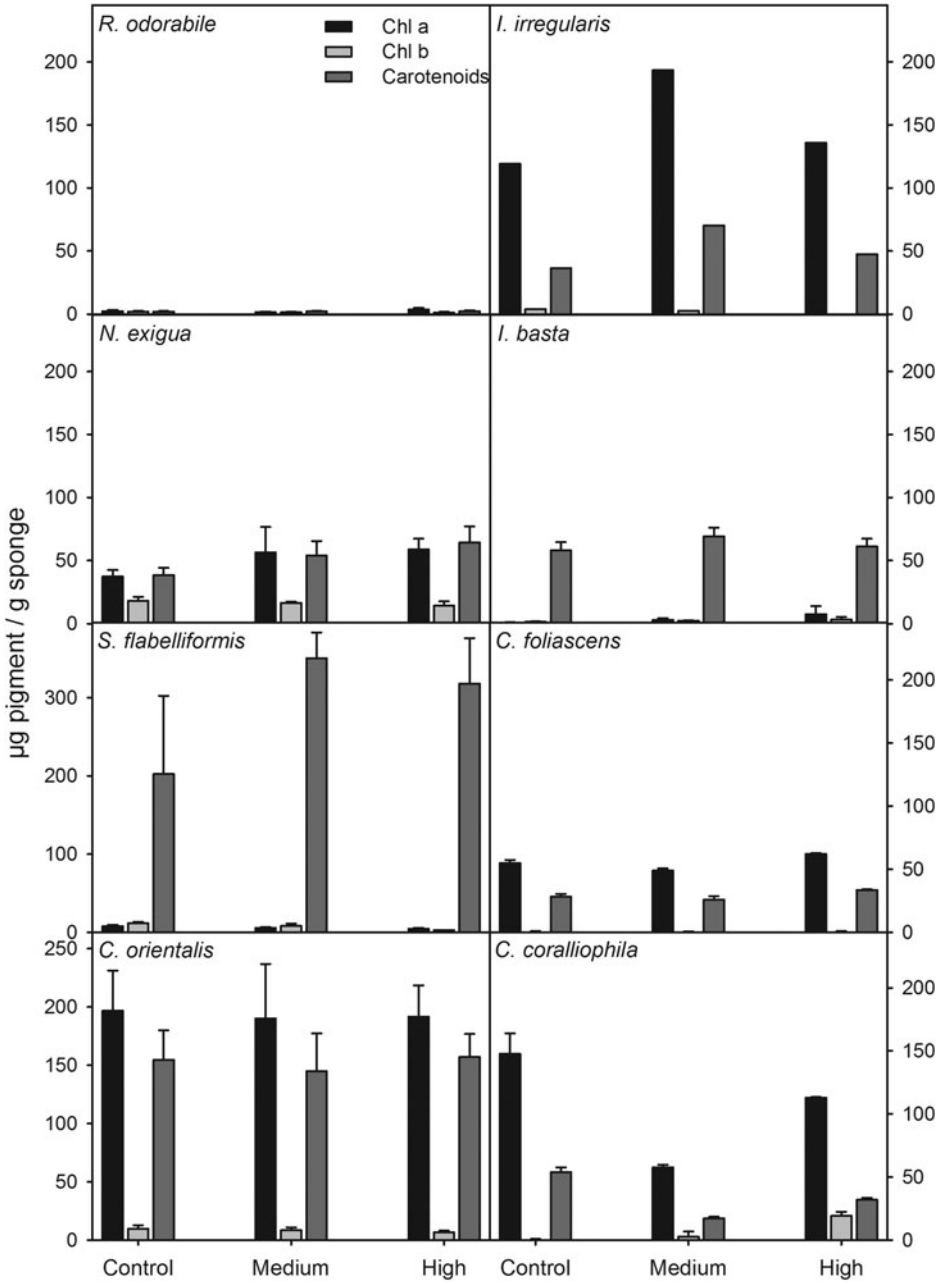


**Fig. 6.** Chlorophylls *a*, *b* and carotenoids (µg pigment/g sponge tissue) for all species at the three sediment treatments (control, medium and high).

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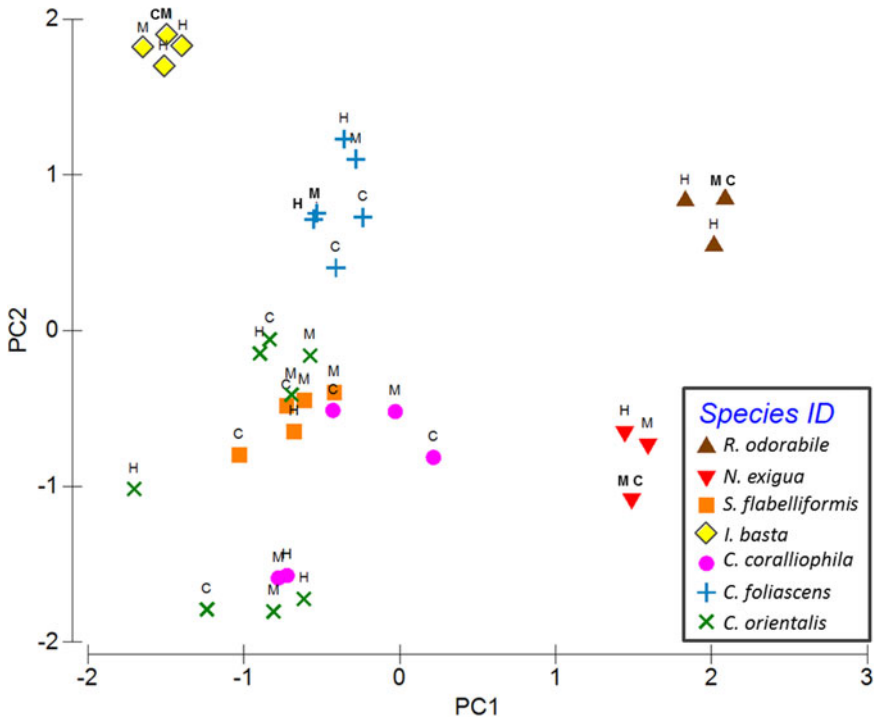


**Fig. 7.** Principal component analysis (PCA) of DGGE banding pattern profiles. Labels correspond to the sediment treatment (C, M and H).

proxy for sedimentation stress in sponges following a short-term sediment pulse. Nevertheless, longer-term experiments with higher levels of replication per species may result in more meaningful conclusions about the effect of sediments on sponge photosynthetic pigment production.

Assessment of microbial symbionts in the studied species revealed generally stable and species-specific communities with some similarity observed within morphologies. Shifts in the microbial communities of sponges have previously been reported in association with temperature and contamination stress (Webster *et al.*, 2001, 2008; Simister *et al.*, 2012a; Fan *et al.*, 2013) although highly stable populations have also been reported in response to nutrient and sediment stress (Luter *et al.*, 2012, 2014; Simister *et al.*, 2012b). Consistent with these latter findings, sedimentation treatments assessed in this study did not cause large shifts in the microbial communities of any species. Small differences between control sponges and sediment-treated sponges were detected in the two photosynthetic species *Carteriospongia foliascens* and *Cymbastela coralliophila* as well as the heterotrophic species *Rhopaloeides odorabile*, although greater replication would be required to determine if these shifts are truly significant. In addition, whilst we don't see the appearance of foreign microbes or the loss of stable microbes associated with sediment treatment, a shift in the relative abundance of the microbes within the host (not detectable using a DGGE approach) may still have functional implications for the holobiont. Further research using next-generation sequencing approaches would help elucidate whether higher sediment concentrations or longer exposure periods would trigger shifts in the sponge-associated microbial communities.

Dredging programmes often last for many weeks or months, possibly exposing benthic organisms to periodically high sediment loads, with sedimentation rates affected by TSS, sediment size and local hydrodynamics. In addition, residual plumes can persist in the area for months before TSS returns to ambient levels. In conclusion, our results show that high sedimentation primarily affected massive, encrusting and wide cup sponge morphologies. However, the sediment concentrations tested in this experiment did not appear to cause shifts in the pigment concentrations or microbial community structure of the sponges. These results indicate that a single short-term pulse of high TSS levels resulting in a sediment deposition rate of 16 mg $cm^{-2}$ could be tolerated by most of the sponge species studied. Nevertheless, the long-term effects of high sedimentation, high TSS and light attenuation on sponges should be assessed before final conclusions on the effect of dredging on sponge communities can be drawn.

**Table 6.** PERMANOVA analysis for each factor separately.

| Source | *df* | MS | Pseudo-*F* | *P* (perm) |
|---|---|---|---|---|
| Sedimentation | 2 | 258.62 | 0.10636 | 0.998 |
| Error | 43 | 2431.7 | | |
| Morphology | 3 | 17,980 | 14.767 | 0.001 |
| Error | 42 | 1217.6 | | |
| Species | 6 | 14,172 | 27.569 | 0.001 |
| Error | 39 | 514.04 | | |

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